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# Antiviral and Antibacterial Polyurethanes of Various Modalities

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**Abstract** We have prepared and characterized a new polyurethane-based antimicrobial material, *N*,*N*-dodecyl,methyl-polyurethane (Quat-12-PU). It exhibits strong antiviral and antibacterial activities when coated (as an organic solution or an aqueous nanosuspension) onto surfaces and antibacterial activity when electrospun into nanofibers. Quat-12-PU surfaces are able to kill airborne Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria, as well as inactivate the enveloped influenza virus (but not the non-enveloped poliovirus).

**Keywords** Antiviral · Antibacterial · Polyurethane · Nanoparticles · Nanofibers

#### Introduction

Contaminated water supplies, ventilation ducts, and household items can serve as reservoirs for pathogenic viruses and bacteria. Likewise, such infectious diseases as influenza are commonly transmitted through surfaces on which aerosols—orally or nasally ejected by already infected individuals—have settled. To prevent the spread of disease through contaminated surfaces, polymeric quaternary ammonium compounds, polyQACs, either covalently attached or painted onto them, have been developed that can act as non-leaching antimicrobial coatings [1–7].

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Immobilized hydrophobic polyQACs based on alkylated polyethylenimine (PEI) exert their antimicrobial action by disrupting cell walls and/or outer membranes of bacteria and fungi [4, 8–13]. The addition of long, hydrophobic chains on polyQACs has been correlated with antibacterial activity. The cationic sites of the polyQACs are adsorbed onto anionic sites of the cell-wall by electrostatic interaction, and subsequently diffuse through the cell wall and bind to the cell membrane. At this stage, the alkyl chains act as a surfactant, disrupting the cytoplasmic membrane, which results in a release of electrolytes and nucleic materials resulting in the cell death [10, 11]. PolyQACs with alkyl chain length between C<sub>6</sub> and C<sub>12</sub> have shown excellent bactericidal activity against both Gram-positive and Gram-negative bacteria [12–14]. In addition, such polyQACs as *N*,*N*-dodecyl,methyl-PEI have been recently reported to inactivate enveloped viruses, including both human and avian strains of influenza virus [15, 16], and disinfect solutions containing non-enveloped poliovirus or rotavirus [17].

To be widely applicable, polyQACs should be robust, chemically diverse, and with modalities readily adjustable to fit specific requirements. In the present work, we have explored antimicrobial materials based on polyurethane (PU), which was selected as a platform due to its ubiquity, versatility, and excellent impact strength and abrasion-resistant characteristics [18], which are particularly advantageous characteristics for the antimicrobial materials that need to be intact for a long period of time. Surfaces coated with such quaternized PU can be processed into nanoparticles and nanofibers with superior antibacterial and antiviral properties.

#### Materials and Methods

#### Materials

*N*-BOC-serinol, 4,4'-methylene-*bis*(phenyl isocyanate) (4,4'-MDI), NaHCO<sub>3</sub>, 1-bromohexane, 1-bromododecane, and 1-iodomethane were from Sigma-Aldrich (St. Louis, MO, USA); diethyl ether from Fisher Scientific (Pittsburgh, PA); anhydrous chloroform, anhydrous *N*,*N*-dimethylformamide (DMF), tetrahydrofuran (THF), and trifluoroacetic acid (TFA, 99 %) from EMD (Gibbstown, NJ, USA). Viruses were obtained from the U.S. Centers for Disease Control and Prevention (CDC) (Wuhan strain of influenza) and the American Type Tissue Collection (ATCC) (Chat strain of poliovirus). Madin–Darby canine kidney (MDCK) and HeLa cells were purchased from ATCC and maintained as previously reported [16, 17]. *Escherichia coli* and *Staphylococcus aureus* bacteria were purchased from ATCC (catalog numbers 6538 and 15597, respectively) and grown in LB broth.

#### Equipment

<sup>1</sup>H FT-NMR spectra were recorded using a Bruker Avance 600 NMR instrument. Water contact angles were measured using a VCA Optima XE instrument (AST Products) at room temperature. UV–VIS spectra were recorded using an UVmini-1240 spectrophotometer (Shimadzu Scientific Instruments). Scanning electron microscopy (SEM) images of polymer-coated surfaces subsequently sputter-coated with gold were obtained using a JEOL 6330F instrument at a 5-kV accelerating voltage. The molecular weights of polymers were determined by gel permeation chromatography (GPC) using a Viscotek GPCmax VE2001 system equipped with a Viscotek I-MBMMW-3078 column and a dual detector (Viscotek 270, differential refractive index and right angle light scattering) and using DMF as a mobile phase. The size distribution of nanoparticles was recorded by a Nicomp 380 ZLS (Particle Sizing Systems, Santa Barbara, CA, USA).



Synthesis of Quaternized Polyurethane (Fig. 1)

Synthesis of PU (Step 1)

*N*-BOC-serinol (2.6 mmol) was dissolved in 5 ml of anhydrous DMF in a 25-ml round-bottom flask and placed in a 90 °C oil bath. One mole-equivalent of 4,4'-MDI was added slowly, and polymerization was carried out for 120 h under a N<sub>2</sub> atmosphere. Then another 2.6 mmol of *N*-BOC-serinol was added, and the reaction mixture was stirred for 24 h. After cooling to room temperature (RT), the reaction mixture was poured into excess of diethyl ether to precipitate the polymer. Following removal of diethyl ether by rotary evaporation, the precipitate was dissolved in 5 ml of DMF and poured into the ether again. The

HN BOC HO OH 
$$^+$$
 OCN  $^+$  A,4'-MDI NCO  $^+$  NCO  $^+$  N-BOC-serinol Step 1  $^+$  90 °C, 144h BOC HN HO OH MHO OH M

Fig. 1 Synthesis of Quat-n-PU. *Step 1*: synthesis of PU by the reaction between N-BOC-serinol and 4,4'-MDI. *Step 2*: removal of BOC groups to result in H<sub>2</sub>N-PU. *Step 3*: synthesis of Quat-n-PU by quaternization with long-chain alkyl bromides; n=6 or 12. See "Materials and Methods" for details



purification process was carried out thrice to remove unreacted components. PU was obtained after drying the precipitate at 45  $^{\circ}$ C under vacuum (98  $^{\circ}$ 4 yield,  $M_{\rm w}$  of 12,200 Da).

# Deprotection (Step 2)

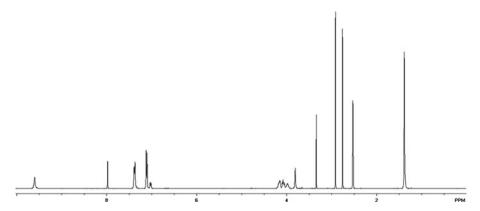
To remove BOC protecting groups and introduce quaternizable amino groups, 1 g of PU was dissolved in 100 ml of chloroform in a 500-ml round-bottom flask. Then 100 ml of TFA was added (50 %, v/v), and BOC deprotection was performed for 1 h at RT. After removing TFA and chloroform by rotary evaporation, the resultant polymer was dissolved in 10 ml of DMF and poured into excess of diethyl ether. The deprotected PU ( $H_2N-PU$ ) was obtained after drying the purified precipitate for 48 h at 45 °C under vacuum (97 % yield,  $M_w$  of 9,128 Da). This experimental  $M_w$  of the deprotected polyurethane is not appreciably different from theoretical  $M_w$ , which indicates that the deprotection step has no adverse effect on urethane linkages.

# Quaternization of PU (Step 3)

 $H_2N$ -PU (1 g) was dissolved in 50 ml of anhydrous DMF in a 100-ml round-bottom flask, and 5 mole-equivalents of either 1-bromohexane or 1-bromododecane was added slowly, followed by 3 mole-equivalents of NaHCO<sub>3</sub>. The alkylation was performed for 120 h at 95 °C under a  $N_2$  atmosphere. Complete quaternization was carried out by subsequently adding 3 mole-equivalents of iodomethane for 24 h at 60 °C. After removing solids by filtration, the reaction mixture was poured into excess of diethyl ether. The precipitate was recovered, dissolved in 20 ml of DMF, and re-precipitated in the ether. This process was repeated thrice to remove excess reagents. Quaternized PU derivatives were obtained after drying the precipitates for 48 h at 45 °C under reduced pressure. We designated quaternized PUs as Quat-n-PU, where n is either 6 or 12 corresponding the number of methylene groups in the 1-bromoalkane used (95 % and 92 % yields were obtained for Quat-6-PU and Quat-12-PU, respectively).

# <sup>1</sup>H NMR Analysis

PU:  ${}^{1}$ H NMR (DMSO, δ in ppm, Fig. 2): 1.41 (C $H_3$ , BOC), 3.97–4.28 [C $H_2$ CH(NBOC)C $H_2$ ], 7.2 (aromatic), 7.48 (OCONH). Deprotected PU:  ${}^{1}$ H NMR (DMSO, δ in ppm, Fig. 3): 4.2–4.3



**Fig. 2** <sup>1</sup>H NMR spectrum of PU. The methyl protons in BOC group were confirmed at 1.41 ppm. The methylene protons adjacent to oxygen in N-BOC-serinol were observed at 3.97–4.28 ppm. The aromatic protons were confirmed at 7.2 ppm. The signal at 7.48 ppm was assigned to a proton in urethane bond



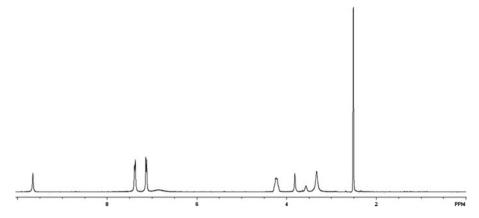
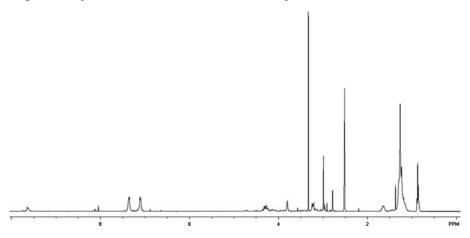


Fig. 3 <sup>1</sup>H NMR spectrum of H<sub>2</sub>N-PU. The signal at 1.41 ppm disappeared indicating a complete deprotection

[C $H_2$ CH(NH<sub>2</sub>)C $H_2$ ], 7.18 (aromatic), 7.4 (OCONH). Quat-12-PU: <sup>1</sup>H NMR (DMSO, δ in ppm, Fig. 4) of Quat-12-PU: 0.9 [NCH<sub>2</sub>(CH<sub>2</sub>)<sub>10</sub>C $H_3$ ], 1.3–1.7 [NCH<sub>2</sub>(C $H_2$ )<sub>10</sub>C $H_3$ ], 3.3 [NC $H_2$ (CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>, NC $H_3$ ], 4.2–4.3 [C $H_2$ CH(NCH<sub>2</sub>(CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>)C $H_2$ ], 7.2 (aromatic), 7.4 (OCONH).

# Preliminary Antibacterial Tests of Quat-n-PU

The preliminary antibacterial tests were carried out in 24-well cell culture plates coated with 100  $\mu$ l of a 5 mg/ml Quat-n-PU solution in methanol. Bacterial suspensions (1×10<sup>4</sup> cells/200  $\mu$ l) in LB broth were added in each well and incubated for 12 h at 37 °C. The antibacterial activity was determined by measuring optical density of a bacterial suspension at 610 nm [19] using a microplate reader (SYNERGY Mx, BioTek). The percent increase in the optical density was calculated by comparing optical densities of bacterial suspensions before and after the incubation. After the first optical density was measured, the suspensions were removed, and the wells were washed thoroughly with distilled water. Subsequent antibacterial tests were repeated using the same procedure. Uncoated 24-well cell culture plates were used as a control.



**Fig. 4** <sup>1</sup>H NMR spectrum of Quat-12-PU. After quaternization, a significant change was observed between 0.5 and 2 ppm. The methyl protons and methylene protons from 1-bromododecane were confirmed at 0.9 ppm and 1.3–1.7 ppm, respectively, indicating successful alkylation with 1-bromododecane



# Sample Preparation

# Solution Coating

For antiviral tests, 50 mg of Quat-12-PU was dissolved in 10 ml of THF, and the solution was sprayed onto the upward-facing side of each of 30 polyethylene slides (25×25 mm) at a flow rate of 10 ml/min. For antibacterial tests, the same solution was sprayed onto the both sides of each of 35 microscope glass slides (10×25 mm) at the same flow rate. Eight layers of Quat-12-PU solution were sequentially deposited, with drying between the coatings. Following drying for 48 h at 45 °C, the coated slides were stored in a desiccator prior to use.

#### Coating with Nanoparticles

Nanoparticles were prepared by dissolving 10 mg of Quat-12-PU in 5 ml of THF. The solution was slowly added to 50 ml of deionized water in an ultrasonic bath (40 kHz; Branson 1510). After removing the THF by rotary evaporation, the nanoparticles were recovered by a centrifugation at 10,000 rpm for 10 min, followed by dispersing them in 10 ml of deionized water with gentle shaking on an orbital shaker for 72 h. After subsequent centrifugation, the Quat-12-PU nanoparticles were obtained by lyophilization. For antiviral test-slide preparation, 10 mg of nanoparticles was dispersed in 10 ml of deionized water and sprayed onto the upward-facing side of each of 15 polyethylene slides (25×25 mm) at a flow rate of 10 ml/min. For antibacterial test-slide preparation, 10 mg of nanoparticles suspended in 10 ml of deionized water was sprayed onto the both sides of 10 microscope glass slides (10×25 mm) at the same flow rate. Nine layers were sequentially deposited; in both cases, a deposited layer was completely dried before applying the next one.

#### Nanofiber Preparation

Quat-12-PU nanofibers were prepared with an electrospinning device [20] consisting of a syringe with an 18-gauge needle, an aluminum collecting board, and a high-voltage supply. For electrospinning, 20 wt.% of Quat-12-PU solution in THF was electrospun at a voltage of 18 kV with a tip-to-collector distance of 15 cm. The flow rate of syringe pump connected to the syringe was set to 100  $\mu$ l/min. The nanofibers were collected on both sides of a microscope glass slide (10×25 mm).

#### Quantitation of Surface Quaternary Amines

The surface density of quaternary ammonium ions was measured using a fluorescein staining test [21]. Samples (10 mm²) coated with Quat-12-PU using either solution or nanoparticulate formulations were dipped into 10 ml of a 1 % fluorescein Na solution in distilled water for 10 min at 37 °C with gentle shaking. After rinsing thrice with distilled water to remove the unbound dye, the samples were placed in 3 ml of a 0.1 % cetyltrimethylammonium chloride solution in which the bound dye was extracted for 20 min at 37 °C with gentle shaking. After adding 10 %v/v of 100 mM of Na phosphate buffer solution (pH 8.0), the absorbance of the aqueous solution at 501 nm was recorded using a spectrophotometer, and the concentration of previously bound fluorescein was determined using the extinction coefficient of 77 mM⁻¹ cm⁻¹. The quaternary ammonium group surface density was calculated assuming that one fluorescein molecule binds to each quaternary ammonium ion on the surface.



#### Antiviral Tests

Polyethylene slides  $(25\times25\text{ mm})$  coated with either a solution or a nanosuspension of Quat-12-PU were used to determine antiviral activity against the enveloped influenza virus (Wuhan strain, A/Wuhan/359/95) and the non-enveloped poliovirus (Chat strain). The slides were placed coated-side up into a polystyrene Petri dish, and  $10~\mu l$  of either a  $3.8\pm1.0\times10^5$  pfu/ml poliovirus solution in Eagle's minimum essential media (EMEM) or a  $5.9\pm1.7\times10^4$  pfu/ml solution of influenza virus in phosphate buffered saline (PBS) was placed in the center of each slide. The virus-containing droplet was sandwiched with a plain polyethylene slide, and an 8-oz weight was placed on the sandwiched slides to spread out the droplet. The same was done for plain control slides (control 2). Viruses were incubated between the slides for 15 min, after which time the slides were separated with tweezers and washed thoroughly with 990  $\mu l$  of EMEM or PBS for poliovirus and influenza virus, respectively. The washings were collected and assayed for infectious viral particles via a plaque assay as previously described [17, 22]. Plaques were counted and compared to those in a control experiment with the viruses never sandwiched between slides (control 1) to determine antiviral activity of the coated slides.

Antiviral Assay of N,N-Dodecyl,Methyl-PEI Coated Slides and Poliovirus Washed with Detergent Solutions

N,N-Dodecyl,methyl-PEI was synthesized as described previously [22], and polyethylene slides (25×25 mm) coated with this polycation were tested for antiviral activities as outlined above for poliovirus, except that in the washing step the viruses were washed off the slide with 990  $\mu$ l of 0.1 % cetyltrimethylammonium chloride in PBS or 0.05 % Tween 80 in 0.5 M NaCl solution. In controls, solutions of viruses were incubated between two plain polyethylene slides and washed with detergent solutions.

#### Antibacterial Tests

The plain and coated slides (five samples of each slide) were incubated with 2 ml of a bacterial suspension ( $10^8$  cells) in a conical tube (Falcon) for 1 h at 37 °C and 300 rpm. One hundred  $\mu$ l was withdrawn from each tube, serial dilutions of the sample were plated onto LB-agar plates, and the number of colonies was counted after incubation overnight at 37 °C. The antibacterial activity was expressed as log-reduction: the difference between the logarithms of viable cells incubated with plain and coated slides.

#### **Results and Discussion**

## Preliminary Antibacterial Tests

The hydrophobic moieties in our PU system were introduced by means of alkylation by either 1-bromohexane or 1-bromododecane, followed by finishing quaternization with iodomethane, to result in Quat-6-PU and Quat-12-PU, respectively (Fig. 1). The optical density of *E. coli* suspensions incubated in 24-well culture plates coated with Quat-6-PU remained unchanged after a 12-h culture while a 3-fold jump in optical density was observed in the suspensions incubated in uncoated plates under the same conditions. These observations indicate that Quat-6-PU coatings are antibacterial (Fig. 5). The same test was conducted after exhaustively washing the coated surfaces with water to test their robustness and no



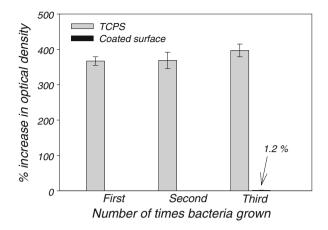
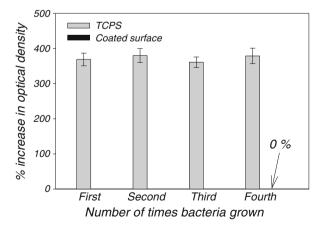


Fig. 5 Antibacterial activity of Quat-6-PU-coated 24-well plates against *E. coli*. No changes in optical density were observed with coated surfaces until after a second washing. A significant increase in the optical density was observed with uncoated surfaces in every test. *TCPS* tissue culture polystyrene

appreciable change in the optical density was observed for either the Quat-6-PU-coated or Quat-12-PU (Fig. 6)-coated plates after repeated thorough washes. Note, however, because of its greater hydrophobicity, Quat-12-PU would be expected to be more resistant to leaching [6], which was indeed the case (see below).

# Water Contact Angle Measurement of Quat-n-PU

To examine the possible leaching of Quat-n-PU from the coated surface after wetting, we measured the changes in dynamic contact angles of polyethylene surfaces coated with Quat-6-PU and Quat-12-PU (Table 1). The advancing ( $\theta_A$ ) and receding ( $\theta_R$ ) contact angles of an uncoated polyethylene slide were 109° and 84°, respectively, and they did not change after a 72-h wetting at room temperature (data not shown). The initial advancing and receding contact angles for a Quat-6-PU coated surface were 92° and 43°, respectively. After the



**Fig. 6** Antibacterial activity of Quat-12-PU against *E. coli*. No changes in optical density were observed with coated surfaces after any washing cycle while a significant increase in the optical density was observed with uncoated surfaces. *TCPS* tissue culture polystyrene



	Before wetting		After wetting	
Polycation	$ heta_{ m A}$	$\theta_{ m R}$	$ heta_{ m A}$	$\theta_{ m R}$
Quat-6-PU	92±3°	43±2°	56±4°	18±3°
Quat-12-PU	106±5°	71±3°	89±2°	61±4°

Table 1 Dynamic contact angles of polyethylene surfaces coated with Quat-6-PU and Quat-12-PU before and after incubation (wetting) in water

surface was incubated in a water bath for 72 h at room temperature, the contact angles dropped to 56° for  $\theta_A$  and 18° for  $\theta_R$  corresponding to a 39 % and 58 % decrease, respectively (Table 1). The initial contact angles of Quat-12-PU-coated surfaces were 106° ( $\theta_A$ ) and 71° ( $\theta_R$ ), i.e., expectedly higher (reflecting a greater hydrophobicity) than those for Quat-6-PU. Importantly, a decrease in contact angles after the aqueous incubation was several-fold smaller for Quat-12-PU than for Quat-6-PU coatings: only 15 % ( $\theta_A$ ) and 14 % ( $\theta_R$ ). These data confirm that the more hydrophobic coating is more resistant to leaching. Based on these findings, we selected Quat-12-PU for further experiments.

## Determination of Coating Cycle by Charge Density of Quaternary Ammonium Ions

Antibacterial activity for polyQACs depends on the surface charge density of quaternary ammonium ions [21]. We hypothesized that the charge density could be increased by applying additional layers of Quat-12-PU onto surfaces. That was found to be indeed the case for surfaces coated with either THF solutions or aqueous nanosuspensions of Quat-12-PU (Table 2). The size of Quat-12-PU nanoparticles used for nanosuspension coating was about 218 nm (Fig. 7). As seen in Table 2, the saturation occurred after eight coating cycles for a solution and nine cycles for a nanosuspension, with only negligible changes in the quaternary ammonium group density on the surface thereafter; these numbers were thus chosen for subsequent antimicrobial tests. Figure 8 depicts SEM images of the surface coated with Quat-12-PU nanoparticles.

# Antibacterial and Antiviral Activity of Quat-12-PU

As seen in Table 3, glass surfaces coated with either a THF solution or an aqueous nanosuspension of Quat-12-PU showed excellent antibacterial activities against waterborne E.

**Table 2** The dependence of the surface charge density of quaternary ammonium ions on the number of coating cycles of Quat-12-PU from its THF solution and an aqueous nanosuspension

	Charge density/cm <sup>2</sup> (×10 <sup>15</sup> )		
Number of coating cycles	Solution	Nanosuspension	
5	4.30	4.91	
6	5.85	6.36	
7	6.11	7.51	
8	6.28	7.58	
9	6.27	7.61	
10	6.28	7.61	
11	n.d.	7.60	



 $<sup>\</sup>theta_A$  and  $\theta_R$  are advancing and receding contact angles, respectively

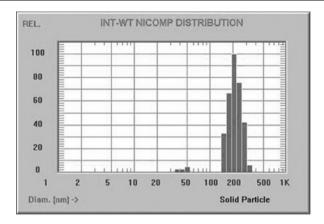


Fig. 7 The size distribution of Quat-12-PU nanoparticles evaluated by dynamic light scattering (DLS). It can be seen that two populations of sizes were observed with mean diameters of 47 nm and 218 nm; the vast majority of the nanoparticles were in the latter group

coli and S. aureus. The log-reduction of S. aureus and E. coli incubated with slides coated with the THF solution was 7.1 and 6.92, respectively. Interestingly the log-reduction of both bacteria incubated with slides prepared by the aqueous nanosuspension was higher than that of the THF solution coated slides: 7.78 and 7.76 for S. aureus and E. coli, respectively. The higher log-reduction might be caused by the higher surface charge density of quaternary ammonium ions on the slides coated with the aqueous nanosuspension as listed in Table 2.

To examine the antiviral activities of Quat-12-PU, polyethylene slides coated with its THF solution or aqueous nanosuspensions were incubated with two distinct types of pathogenic viruses: influenza (enveloped) and poliovirus (non-enveloped). As seen in Table 4, regardless of the mode of coating, the resultant surfaces were completely lethal to the human Wuhan strain of influenza virus. Presumably, the hydrophobic Quat-12-PU coatings disrupt the lipid envelope of the virus protecting its RNA, as was previously demonstrated for *N*,*N*-dodecyl,methyl-PEI coatings [16]. However, Quat-12-PU-coated surfaces failed to inactivate poliovirus (Table 4).

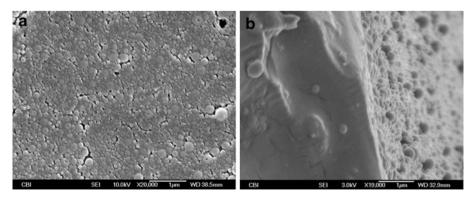


Fig. 8 SEM images of a polyethylene surface coated with Quat-12-PU nanoparticles. The top view (a) and cross-section (b) of the surface coated with an aqueous nanosuspension



**Table 3** The log-reduction of cells inoculated with Quat-12-PU-coated (using either a THF solution or an aqueous nanosuspension) glass surfaces

Slide coated with				
THF solution		Aqueous nanosuspension		
S. aureus	E. coli	S. aureus	E. coli	
7.1±0.04	6.92±0.03	7.78±0.05	7.76±0.03	

**Table 4** Antiviral activities of uncoated and Quat-12-PU-coated (using either a THF solution or an aqueous nanosuspension) polyethylene slides

Virus	No slide	Uncoated slide	Slide coated with a solution	Slide coated with a nanosuspension
Influenza (pfu/ml) Poliovirus (pfu/ml)	$(5.9\pm1.7)\times10^4$ $(3.8\pm1.0)\times10^5$	$(2.2\pm0.3)\times10^4$ $(1.7\pm0.4)\times10^5$	0 (1.6±0.4)×10 <sup>5</sup>	0 $(1.4\pm0.5)\times10^5$

**Table 5** The recovery of poliovirus by washing uncoated and *N,N*-dodecyl,methyl PEI-coated polyethylene slides with the detergents cetyltrimethylammonium chloride (CTAC) and Tween 80<sup>a</sup>

Treatment	Viral titer (pfu/ml)
Control virus solution	$(1.9\pm0.7)\times10^5$
Plain slide, CTAC wash	$(1.8\pm0.4)\times10^5$
Plain slide, Tween 80 wash	$(1.6\pm0.7)\times10^5$
Coated slide, CTAC wash	$(0.13\pm0.1)\times10^5$
Coated slide, Tween 80 wash	$(0.6\pm0.1)\times10^5$

<sup>&</sup>lt;sup>a</sup> Plain or coated slides were incubated with poliovirus and washed with a detergent

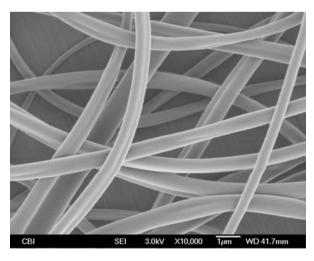


Fig. 9 The SEM of Quat-12-PU nanofibers electrospun from a 20 wt.% solution of Quat-12-PU in THF



**Table 6** The log-reduction of cells incubated with slides to which waterborne bacteria were applied on top of a Quat-12-PU nanofiber coating

S. aureus	E. coli
7.53±0.05	7.49±0.02

Since in our previous experiments *N*,*N*-dodecyl,methyl-PEI coatings disinfected solutions of this virus [17], we examined herein whether polioviruses in that case were genuinely inactivated or merely adhered to the polycation-coated surfaces. To this end, following incubation with the virus, we washed the *N*,*N*-dodecyl,methyl-PEI-coated slides with two types of detergents, cationic cetyltrimethylammonium chloride and non-ionic Tween 80, in an attempt to recover polioviruses that reversibly adhered to the surface. Indeed, analysis of the washings for both detergents revealed the recovery of most of the infectivity lost from the original solution (Table 5), pointing to the adsorptive mechanism of the disinfection. That polioviruses adhere to *N*,*N*-dodecyl,methyl-PEI-coated surfaces but not to Quat-12-PU-coated surfaces may be attributed to the different chemical makeups and consequent coated surface properties of these two polymers.

To expand the utility of Quat-12-PU beyond surface coatings, we processed the polycationic polymer into nanofibers using an electrospinning method [20]. Specifically, solutions of Quat-12-PU in THF were electrospun to obtain continuous and uniform nanofibers seen in Fig. 9. Like with Quat-12-PU-coated surfaces tested in this study, these nanofibers were found to exhibit excellent antibacterial activities against both *E. coli* and *S. aureus* (Table 6): the log-reductions were close to 7.5 for both cells. With polymeric nanofibers having potential to be used in fabrics, filters, and medical devices [23, 24], those made from Quat-12-PU would have an added benefit of being antimicrobial.

#### Conclusions

We have created a novel antiviral and antibacterial polyurethane, Quat-12-PU, that was easily processed into solutions, nanoparticles, and nanofibers. However created, Quat-12-PU surfaces efficiently inactivated both *S. aureus* and *E. coli* bacteria, as well as influenza viruses. Given the high efficiency of microbicidal activity and easy processibility, it may lead to a broad range of applications.

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